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LABORATORY AND FIELD VARIATION IN HUFA ENRICHMENT OF *ARTEMIA* NAUPLII

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Introduction

The development of suitable bioencapsulation techniques for live food has resulted in a major break-through in the hatchery production of marine fish fry in the eighties. This allowed the incorporation of supra-natural levels of DHA 22:6n-3 and EPA 20:5n-3 in the gut and body tissues of rotifers and *Artemia* prior to their being fed to fish larvae that have a high requirement for these essential fatty acids. In *Artemia*, the most commonly applied boosting technique is to use commercial fish oil emulsions (Selco range, INVE Aquaculture NV, Belgium) which are added twice at concentrations of $0.3 g.l^{-1}$ to the *Artemia* containers, 0h and 12h after hatching (Léger et al., 1987). In this way, it is generally accepted that levels of 35 and $60 mg.g^{-1}$ DW of total n-3 HUFA (highly unsaturated fatty acids) can be obtained in 24-h-old nauplii using Selco or Superselco (INVE Aquaculture NV, Belgium), respectively, which should be sufficient to meet specific predator's requirements. However, although this technique is claimed to be standardised, no attempts have been made to check the variability of enrichment under different conditions. Since such information is considered important both for the culturist and the researcher who wants to identify exact quantitative requirements for the fish larval stages, the ICES Working Group on Mass Rearing of Juvenile Marine Fish recommended studies to evaluate the technique under laboratory and field conditions (ICES, 1993).

Materials and methods

Laboratory testing was performed at the *Artemia* Reference Center, Belgium. Standard conditions for *Artemia* hatching and enrichment were set as follows: Great Salt Lake (GSL) *Artemia* cysts (INVE Aquaculture NV, Belgium) were incubated at densities of $1 g.l^{-1}$ in 40 l cylindroconical hatching containers (35ppt salinity; 28°C) under optimal conditions (Sorgeloos et al., 1986) for 24h. Freshly-hatched nauplii were harvested using a submerged filter of 150µm, reincubated under identical conditions

at a density of 250.ml^{-1}, and 0.3g.l^{-1} of Superselco was added. After 12h a second identical amount was administered to the enrichment tanks. After 24h enrichment, samples (± 2g WW) of the *Artemia* metanauplii were taken, rinsed in tap water, and stored in glass vials at -25°C until analysis. This standard set-up was used 10 times over a period of 2 months in 1994 by the same person (Table Ia), as well as by various people over a period of respectively 2 months in 1994 (Table Ib) and 2 years (1992-93; Table Ic).

For field verification, samples of enriched *Artemia* were taken from three hatcheries of seabream *Sparus aurata* in the south of Spain. The hatcheries were requested to sample enriched *Artemia* produced according to their in-house procedure at the moment of the first feeding of the day (to avoid effects of storage). Three samples were requested with at least 3 days between the sampling (to have three samples independent in time). Samples were well rinsed to remove all possible fat containing contaminants, frozen immediately after sampling, and transported in dry ice to the laboratory for analysis.

The fatty acid composition was determined using gas chromatography. Samples from the laboratory testing (Table I) were analysed following the ICES adopted procedure of Coutteau and Sorgeloos (1995). For the field samples FAME (fatty acid methyl esters) were prepared according a modified protocol of Lepage and Roy (1984). It implicates a direct acid catalised transesterification without prior extraction of total fat, on dry sample amounts ranging from 10 to 30mg; an internal standard 20:2n-6 was added prior to the reaction. Fatty acid values are expressed as area % or mg.g^{-1} DW of FAME.

Results and discussion

The results of the laboratory testing reveal a high variability in essential fatty acid composition of the *Artemia*, be it enriched by the same person (Table Ia) or by various people (Table Ib). There is even no reduction in variability when one person handled the standard enrichment procedure instead of different people: e.g. total n-3 HUFA varied from 15 to 28% or 22 to 68mg.g^{-1} DW and 16 to 30% or 32 to 64mg.g^{-1} DW, respectively. A similar variability is noted in the sampling campaign over a longer period of time (2 years).

The results of the field study indicate that the average n-3 HUFA content of enriched *Artemia* varies among hatcheries from 2.8 to 4.7% on a dry weight basis (Table II). Also, variability among batches of enriched *Artemia* is different. Only hatchery C manages to keep the variability in the n-3 HUFA content after enrichment below 9% (CV of the data for n-3 HUFA in mg.g^{-1} DW), whereas a higher variability is found in the other hatcheries (e.g. CV of 22% for hatchery B, two very different samples for hatchery A).

Table I. EPA, DHA and total n-3 HUFA composition of *Artemia* nauplii enriched using a standard procedure; Ia: applied by the same person (n =10), Ib: applied by different people during a 2m-period (n=5) or Ic: during a 2-year-period (n=13).(Mean and standard deviation)

	EPA		DHA		n-3 HUFA	
	Area %	mg.g^{-1}	Area %	mg.g^{-1}	Area %	mg.g^{-1}
Ia						
Mean	13.8	24.2	7.1	12.9	23.5	41.9
sd	2.2	5.7	2.5	6.5	4.5	13.1
Ib						
Mean	14.5	27.0	6.2	11.3	23.3	43.0
sd	4.1	9.9	0.9	2.6	5.1	12.9
Ic						
Mean	16.7	35.7	7.8	17.0	26.7	57.4
sd	2.3	7.6	2.2	5.8	4.8	14.2

Table II. EPA, DHA and total n-3 HUFA composition of *Artemia* nauplii enriched according to the in-house method in three seabream hatcheries. (Mean and sd from n samples)

Hatchery	EPA		DHA		Sum n-3 HUFA	
	Area %	mg.g^{-1} DW	Area %	mg.g^{-1} DW	Area %	mg.g^{-1} DW
A (n=2)	9.9 ±4.0	20.3 ±11.2	3.8 ±2.5	8.1 ±6.3	16.1±7.1	33.2 ±19.7
B (n=3)	10.5 ±1.1	15.9 ±5.4	5.9 ±2.4	8.1 ±1.4	20.0±5.8	28.5 ±6.4
C (n=3)	14.2 ±0.8	29.1 ±2.3	6.1 ±0.6	12.6 ±1.5	12.6±1.5	46.6 ±4.0

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